# Abstracts

## The British Society for Cardiovascular Research Spring Meeting 2007

## Emerging therapeutic targets and technologies for the treatment of cardiovascular disease

## Oral communications

### 001 DEVELOPMENT OF A MOUSE ISOLATED HEART MODEL OF ISCHAEMIA INDUCED VENTRICULAR FIBRILLATION

C. L. Stables, M. Avkiran, M. J. Curtis. *Cardiovascular Division, King's College London, St Thomas' Hospital, London, UK*

Functional genomics could be used to identify novel modulators of ischaemia induced lethal arrhythmias, but this application has been limited by the infrequency of ventricular fibrillation (VF) in the wildtype murine heart. The aim of this study was to develop a robust mouse isolated-heart model of ischaemia/reperfusion induced VF. The initial strategy was to reintroduce catecholamines and angiotensin II (A-II), putative arrhythmogens in vivo that are ordinarily absent in perfused heart preparations. Hearts from male T/O mice were Langendorff perfused with control solution (standard Krebs modified to contain 2.4 mM $Ca^{2+}$ and 3 mM $K^{+}$) before being perfused with a test solution containing catecholamines (noradrenaline 313 nM, adrenaline 75 nM) and/or A-II (100 pM), or control (n = 10 per group). Hearts were then subjected to 30 min regional ischaemia by ligation of the left main coronary artery, followed by reperfusion. The ECG was monitored throughout. Catecholamine perfusion significantly increased VF incidence during ischaemia (5/10 *vs* 0/10 hearts, $p<0.05$) and during reperfusion (5/10 *vs* 0/10 hearts, $p<0.05$). Catecholamine perfusion also had typical haemodynamic effects ($p<0.05$ *vs* controls), increasing heart rate from $380\pm15$ to $457\pm25$ beats/min and coronary flow from $18\pm2$ to $30\pm3$ ml.min$^{-1}$g$^{-1}$ (values 14 min before ischaemia). A-II had no effect on VF susceptibility or on the VF priming effect of catecholamines. In conclusion, physiological catecholamine supplementation converts the VF resistant mouse perfused heart into a viable bioassay for investigating modulation of ischaemia induced VF, using functional genomics.

This work was funded by a British Heart Foundation PhD studentship.

### 002 MODULATION OF NADPH OXIDASE ACTIVITY AND REDOX SIGNALLING BY THE ADENOSINE A2A RECEPTOR ANTAGONIST SCH58261

D. Ribé, J.-M. Li. *Cardiovascular Research Theme, SBMS, University of Surrey, Guildford, UK*

It was discovered recently that cardiac tissues express constitutively a multicomponent NADPH oxidase which generates reactive oxygen species (ROS). The ROS thus produced serve as secondary messengers in redox signalling pathways and participate in the regulation of cardiac development and cardiovascular function. In this study, we investigated the role of NADPH oxidase and ROS in mediating cardiac adenosine A2A receptor (A2AR) signalling in wildtype mice on a CD1 background. Mice were treated by intraperitoneal injection with the A2AR antagonist SCH58261 (10 mg/kg body weight) in phosphate buffered saline (PBS) or with PBS only (as control). Nine mice were used in each treatment group. Hearts were harvested 90 minutes after injection and examined for acute A2AR blockade induced changes in NADPH oxidase activity ($O_2^-$ production) and the activation of MAPK signalling pathways. Compared with control hearts, basal ROS production by cardiac tissue showed no significant changes after SCH58261 treatment. However, SCH58261 treatment resulted in a $48\pm8$% reduction in NADPH (100 μM) dependent $O_2^-$ production ($p<0.05$). The ROS production was completely abolished by adding tiron (an $O_2^-$ scavenger) and inhibited by apocynin (an NADPH oxidase inhibitor), but not by oxypurinol (a xanthine oxidase inhibitor), rotenone (a mitochondrial oxidase inhibitor), or L-NAME (a nitric oxide synthase inhibitor). Accompanied by the reduction of NADPH oxidase activity, there was a $54\pm28$% reduction in JNK phosphorylation in SCH58261 treated hearts as detected by phospho-JNK specific antibody ($p<0.05$). There were no significant changes in ERK1/2 and p38 MAPK phosphorylation between control and SCH58261 treated hearts. These results indicate that NADPH oxidase and its product ROS are functionally involved in adenosine signalling through the A2AR via the modulation of redox sensitive JNK signalling pathways. Antagonists to A2AR such as SCH58261 may have therapeutic applications in the treatment of diseases related to cardiac oxidant stress.

### 003 ADAM 15 IS ESSENTIAL FOR REGULATED ANGIOGENESIS AND VEGF SIGNALLING TO AKT THROUGH PROTEOLYTIC PROCESSING OF THE UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR (uPAR)

S. Rahman[1], A. Lundkvist[2], C. Xu[1], R. Sumathipala[1], T. Fujisawa[1], E. Wijelath[3], Y. Patel[1], G. Kanaganayagum[1], M. Sobel[3], H. Gerhardt[2]. *[1]Laboratory of Thrombosis and Vascular Remodelling, Division of Cardiovascular Medicine, King's College London School of Medicine, London, UK; [2]Vascular Biology Laboratory, Cancer Research UK, London, UK; [3]Department of Surgery, Division of Vascular Surgery, Veterans Affairs Puget Sound Health Care System and the University of Washington School of Medicine, Seattle, USA*

Angiogenesis is a complex process whereby neocapillaries sprout from pre-existing vessels in response to hypoxia. ADAM 15 (metagidin) is a member of the mammalian disintegrin-metalloprotease family that is highly expressed in vascular cells and is known to bind to integrins expressed on endothelial cells. However, the biological function of ADAM 15 remains unclear, as null mice show no discernible developmental abnormalities but appear to display impaired pathological angiogenesis (Horiuchi K, *et al. Mol Cell Biol* 2003;**23**:5614–24). In the present study we show that ADAM 15 is essential for regulated angiogenesis. Impairing ADAM 15 function in the murine retina promoted the development of an abnormal vascular plexus comprising of vessels displaying reduced perfusion, patency, and branching. Under these conditions, VEGF induced endothelial cell survival, proliferation, and Akt activation was severely impaired, whereas cell migration, associated urokinase activity, and uPAR antigen levels were raised. In contrast, stable expression of ADAM 15 in monocytic U937 cells, a high uPAR expressing cell line, promoted severe loss of surface uPAR antigen levels and urokinase activity. In addition, recombinant ADAM 15 but not ADAM 12 metalloprotease domain cleaved recombinant uPAR in a purified in vitro assay. These studies establish a role for ADAM 15 in regulating angiogenesis through mechanisms promoting VEGF signalling to Akt and via the negative regulation of the plasminogen activation system through the proteolytic processing of uPAR.

### 004 HAEM OXYGENASE-1 IS A NEGATIVE REGULATOR OF SOLUBLE FLT-1 AND SOLUBLE ENDOGLIN

S. Ahmad, M. Cudmore, B. Al-Ani, K. Chudasama, T. Fujisawa, P. Hewett, A. Ahmed. *Department of Reproductive and Vascular Biology, Institute of Biomedical Research, University of Birmingham, UK*

**Background:** Preeclampsia is characterised by hypertension, proteinuria, and dysregulated angiogenesis. Soluble Flt-1 (sFlt-1) and endoglin (sEng) are increased in preeclampsia, and their co-administration to rats elicits severe preeclampsia-like symptoms. Haem oxygenase-1 (HO-1) and its metabolite, carbon monoxide (CO), exert protective effects against oxidative stimuli in several organs. As HO-1 is downregulated in preeclampsia, an investigation was undertaken into its role in the regulation of sFlt-1 and sEng.
**Results:** Adenoviral overexpression of vascular endothelial growth factor (VEGF) induced a threefold increase in plasma sFlt-1 levels in mice. VEGF

mediated sFlt release required VEGF receptor-2 activation as SU1498 blocked its production. Endothelial cells overexpressing HO-1 or pre-treated with CO releasing molecule (CORM-2) or CO gas decreased the basal and VEGF and interferon-$\gamma$ (INF$\gamma$) induced sFlt-1 release; CO inhibited VEGF induced sFlt-1 by inhibiting VEGFR-2 phosphorylation. Treatment of villous explants or endothelial cells with the HO inhibitor tin protoporphyrin-IX potentiated VEGF and INF$\gamma$ induced sFlt-1 expression, as did siRNA knockdown of HO-1. Consistent with these findings, levels of sFlt-1 in plasma and tissue lysates in HO-1 deficient mice were significantly higher than in wildtype mice. sEng release was greatly increased in preeclamptic placental explants, and administration of AdHO-1 significantly decreased the basal, IFN$\gamma$, and tumour necrosis factor $\alpha$ induced sEng from endothelial cells, while siRNA to HO-1 increased basal sEng and potentiated cytokine induced production. Vitamins C and E had no effect on sFlt-1 or sEng release or HO-1 protein expression, consistent their lack of efficacy in preeclampsia, whereas statins inhibited sFlt-1 release from endothelial cells and explants and upregulated HO-1.
**Conclusions:** This study establishes the HO-1/CO pathway as a negative regulator of cytokine induced sFlt-1 and sEng release. As raised levels of sFlt-1 and sEng are associated with the clinical symptoms of preeclampsia, the findings provide compelling evidence for a protective role of HO-1 in pregnancy and identify a novel target for the treatment of preeclampsia.

# Poster presentations

## 005 A STUDY OF CELL CYCLE AND STEM CELL MARKERS TO IDENTIFY THE FACTORS RESPONSIBLE FOR THE CARDIAC REGENERATION OBSERVED IN MRL MICE

F. L. Moseley[1], M. E. Faircloth[2], W. Lockwood[1], M. S. Marber[2], K. A. Bicknell[1], G. Brooks[1]. [1]*School of Pharmacy, The University of Reading, UK;* [2]*The Rayne Institute, Kings College London, St Thomas' Hospital, London, UK*

The ability of myocardial tissue to undergo repair following injury is restricted. Subsequently, the damaged area is replaced by scar tissue, impairing cardiac function. The MRL mouse strain shows an exceptional regenerative capacity, demonstrating complete healing following a through-and-through ear punch wound. Repair of the myocardium and restoration of heart function have also been observed in these mice following cryoinjury. Both increased cardiomyocyte proliferation and a fetal liver stem cell population have been implicated in this regenerative capacity. Our study aimed to identify the mechanisms facilitating myocardial repair in MRL mice in order to identify potential therapeutic targets in non-regenerative species. Changes in expressions of specific cell cycle regulators that might account for regeneration (CDKs 1, 2, 4, and 6; cyclins A, E, D1, and B1; p21, p27, and E2F5) were compared by immunoblotting in MRL and C57BL/6 ventricles during development. No differences were observed. The effects of injury then were investigated in adult tissues 4 weeks after coronary artery ligation. No differences were observed in infarct size, cardiac function, or contractility between MRL and C57BL/6 mice. However, preliminary histological data suggested that C57BL/6 infarcts are more fibrotic than MRL. Finally, the stem cell markers c-kit, Sca-1, and CD34 were investigated by western blotting and immunofluorescence in fetal liver tissue from MRL and C57BL. Again, no differences were found. Our results eliminate the possibility of intrinsic differences in cell cycle control or stem cell populations in MRL mice. We cannot discount the fact that different methods of injury, such as cryoinjury or coronary artery ligation, might stimulate alternative cellular responses and these remain to be identified.

## 006 THE CYCLIN DEPENDENT KINASE INHIBITOR P27 PLAYS A CRUCIAL ROLE IN REGULATING INDUCTION OF CARDIOMYOCYTE HYPERTROPHY IN VIVO

K. A. Bicknell, I. Kavanagh, W. Lockwood, S. T. Lynch, G. Brooks. *School of Pharmacy, University of Reading, UK*

Cardiac hypertrophy is an adaptive process that occurs in response to increased cardiac demand. However, in certain situations cardiac hypertrophy can be maladaptive and can lead to the development of heart failure. We previously showed that the induction of hypertrophy involves a transient downregulation of the expressions and functions of CDK inhibitors p21 and p27. In the present study, we investigated further the specific role played by p27 in modulating cardiac hypertrophy in vivo. Left ventricular hypertrophy was induced by abdominal aortic constriction (AC) in p27 null and wildtype mice. Assessments of heart weight to body weight (HW/BW) ratios and cardiomyocyte size were made 1, 3, and 7 weeks post-AC or post-sham surgery. HW/BW ratios increased significantly at 3 weeks post-AC compared with controls in both wildtype ($7.3\pm0.4$ *vs* $5.2\pm0.1$ mg/g) and p27 null mice ($7.8\pm0.7$ *vs* $5.7\pm0.2$ mg/g). Consistently, a significant increase in cardiomyocyte size was observed 3 weeks post-AC in wildtype ($131\pm6\%$ *vs* $100\pm6\%$ cell size) and p27 null mice ($141\pm9\%$ *vs* $100\pm8\%$ cell size). Although a significant increase in HW/BW ratio also was observed in p27 null mice one week post-AC compared with controls ($6.8\pm0.2$ *vs* $5.7\pm0.3$ mg/g), no increase in HW/BW ratio was seen at this time point in wildtype hearts when compared with controls. Moreover, there was no difference between HW/BW ratio at 3 or 7 weeks post-AC in p27 null or wildtype mice, suggesting that the hypertrophic response was maximal at 3 weeks post-surgery in our model. Taken together, these data suggest that p27 regulates the early events that control the induction of cardiac hypertrophy and thus that therapeutic strategies targeting its expression might prove valuable to prevent detrimental cardiac hypertrophy that can lead to heart failure.

## 007 CARDIAC DIFFERENTIATION IN XENOPUS REQUIRES THE CDK-INHIBITOR, P27XIC1

M. Movassagh, A. Philpott. *University of Cambridge, Division of Cardiovascular Medicine, Centre for Clinical Investigation, Addenbrooke's Hospital, Cambridge, UK*

In vertebrate cardiac development, the relation between cardiomyocyte differentiation and withdrawal from the cell cycle remains undetermined. CDK inhibitors (CDKIs) are known to be instrumental in cell cycle withdrawal, but their role in myocyte differentiation has not been investigated. Here, we investigated the role of the most prominent Xenopus CDKI, p27Xic1, in cardiac differentiation. During embryonic cardiac development in Xenopus, we observed a fourfold increase in the total number of differentiated myocytes with a small but consistent percentage undergoing mitosis, from stage 29/30 ($1.70\pm0.51\%$) up to stage 41 ($1.88\pm0.28\%$). Incubating Xenopus embryos in media containing hydroxyurea and aphidicolin led to 50-fold reduction in the number of mitotic cells; however, this cell cycle inhibition did not affect cardiac differentiation significantly. These results suggest that cardiac differentiation can occur independent from cell cycle progression. Furthermore, we confirmed p27Xic1 expression in the embryonic Xenopus heart. Upon microinjection of a translation blocking p27Xic1 morpholino (Xic1Mo), 76% of embryos showed a reduction in the area expressing cardiac differentiation markers, whereas 22% showed a similar phenotype following injection with control morpholino (ConMo). To demonstrate specificity for loss of p27Xic1, we coinjected p27Xic1 RNA, which resulted in a partial rescue where the percentage of embryos with normal expression of differentiation markers was doubled from 24% to 57%. Antibody staining of sections from Xic1Mo injected embryos showed half the number of differentiated cardiomyocytes ($310\pm175$) compared with CTRMo injected embryos ($639\pm192$). As our data suggest that p27Xic1 is specifically required for cardiac differentiation, we investigated whether this was dependent on its ability to arrest the cell cycle. Although full length p27Xic1, deletion N-terminal (1-96), C-terminal (97-210), and (35-96)-p27Xic1 constructs could all arrest the cell cycle, only the full length and the N-terminal construct, and *not* the C-terminal or (35-96)-p27Xic1 constructs, could rescue the Xic1Mo phenotype. Our data suggest that the N-terminus but not the C-terminus of p27Xic1 is important in Xenopus cardiac differentiation and this is distinct from its ability to arrest the cell cycle.

## 008 THE ROLE OF REACTIVE OXYGEN SPECIES AND NADPH OXIDASE IN ENDOTHELIAL CELL CYCLE REGULATION

V. T. George, J.-M. Li. *Cardiovascular Research Theme, SBMS, University of Surrey, UK*

Reactive oxygen species (ROS) have been found to play an important role in the regulation of endothelial cell function but the mechanisms involved are not clear. We investigated the time dependent relation between the levels of ROS production and cell cycle progression in endothelial cells maintained (0, 12, 24, 36, and 48 h) in 10% fetal calf serum (FCS) medium (proliferation) and compared with cells under serum starvation (growth arrest). Bovine arterial endothelial cells (BAEC) and human dermal microvascular endothelial cells (HMEC1) were used. In a fully confluent culture (quiescent, G0/G1 $\sim$70%), the basal level of ROS production was low in both endothelial cell lines as detected by lucigenin (5 $\mu$M) chemiluminescence. When these cells (70% confluence) were cultured in

10% FCS and underwent full cell cycle progression, the ROS levels steadily increased up to 24 h of culture (~1.25-fold of the level at 0 h), after which the ROS production decreased while the cell cycle slowed down. In contrast, when cells were subjected to nutrient deprivation (0.2% FCS) and gradually withdrew from the cell cycle, the levels of ROS production showed no significant change up to 24 h of starvation. At 36 h of serum starvation, the levels of ROS production were dramatically increased (~2.15-fold of the 0 h level). High levels of ROS production were accompanied by an increase in cellular apoptosis (~5%) as detected by both trypan blue exclusion and propidium iodide labelled flow cytometry. The high ROS production could be completely inhibited by tiron ($O_2^-$ scavenger) and apocynin (NADPH oxidase inhibitor), but not by oxypurinol (xanthine oxidase inhibitor) or L-NAME (nitric oxide synthase inhibitor), suggesting that the enzymatic source of ROS generation was NADPH oxidase. These data indicate that the levels of ROS produced by NADPH oxidase are functionally involved in cell cycle regulation. A moderate increase in ROS generation is required for serum induced cell growth and proliferation, whereas the activation of NADPH oxidase and the high level of ROS production in response to serum starvation contribute to oxidative stress and cell apoptosis.

## 009 AWARENESS OF COMPLEMENTARY AND ALTERNATIVE MEDICINE IN CARDIOVASCULAR PATIENTS: A CAUSE FOR CONCERN

H. Mattar[1], K. Zacharias[2], A. Afzal[1], N. Carter[1], A. Bakhai[2]. *[1]Division of Clinical Developmental Sciences, St George's University of London; [2]Barnet General Hospital, London, UK*

**Background:** Use of herbal medicines as well as other forms of complementary and alternative medicine (CAM) by cardiovascular patients is increasing. Recent data indicate that there are potentially serious interactions between some commonly used herbal remedies and conventional cardiovascular drugs. Few contemporary data are available on the incidence of such potential interactions or awareness among these patients.
**Aim:** To estimate the incidence of this potential interaction among cardiac inpatients and outpatients, using a survey.
**Design:** 177 cardiovascular patients who presented to Barnet General Hospital were surveyed for this study. Each patient completed a questionnaire with an interviewee regarding their state of CAM usage.
**Result:** Around one third of patients participating in the study used some kind of CAM. Of these, more than half did not inform their health professionals. When asked if they discussed this issue with their doctor, 60% of patients felt it was irrelevant to the health professionals. In around 20% of CAM users there was a significant possibility that the treatments could interact with their conventional cardiovascular drugs. The most common herb–drug interactions were between garlic and warfarin (17%) and between grapefruit juice and statins (17%). The antiplatelet properties of garlic can increase the risk of spontaneous haemorrhage if it is taken with warfarin. Grapefruit juice can induce liver enzymes that metabolise statins, which decrease the half life of these drugs and reduce their efficacy.
**Conclusion:** CAM use is high among cardiac patients. It is often not inquired about or volunteered by the patient and may lead to interactions with conventional drugs. These initial survey data show how important it is to raise awareness of CAM intake among patients and their physicians.

## 010 STRUCTURE–FUNCTION RELATIONS OF POLYPHENOLS AND THE MODULATION OF PLATELET FUNCTION AND SIGNALLING

B. Wright[1], J. A. Lovegrove[2], J. M. Gibbins[1]. *Schools of Biological Sciences[1] and Food Biosciences[2], University of Reading, UK*

It is well established that a polyphenol-rich diet beneficially modulates risk factors involved in cardiovascular disease. Current evidence for the mechanisms underlying these effects provides only a superficial understanding of how polyphenols function in vitro and in vivo. Polyphenols are structurally diverse low molecular weight compounds that are produced as secondary metabolites in plants. The largest and best studied group of polyphenols, the flavonoids, are further divided into subgroups: flavonols, flavan-3-ols, flavanones, and flavones. Other groups include the stilbenes, the hydroxycinnamic acids, and the hydroxybenzoic acids. The connection between thrombosis and cardiovascular disease has motivated research investigating the effects mediated by polyphenols on platelet function and signalling. Much of this work shows a clear relation between the polyphenol structure and their inhibitory effects. To gain a more thorough understanding of this correlation, the effects of structurally related compounds (flavonols, flavan-3-ols, flavones, and flavanones) and unrelated stilbene compounds (hydroxybenzoic acids and hydroxycinnamic acids) were investigated. Collagen-stimulated platelet aggregation, serotonin secretion, global tyrosine phosphorylation, and phosphorylation of Syk and PLC$\gamma$2 were inhibited in a dose dependent manner (IC50: 5.3–3.1 mM). The order of potency established was flavonols = flavones = stilbenes > flavanones > flavan-3-ols > hydroxycinnamic acids > hydroxybenzoic acids. The number of hydroxyl groups on the B ring and the absence or presence of the C2–C3 double bond and the C4 carbonyl group on the C ring determined the potency of the flavonoids. In vivo, flavonoids are methylated, sulphated, or glucuronidated. Therefore the metabolites generated in vivo are the forms of these compounds that are most likely to produce a physiological effect. As such, the effect of polyphenol metabolites on collagen stimulated platelet aggregation, serotonin secretion, global tyrosine phosphorylation, and phosphorylation of Syk and PLC$\gamma$2 were investigated. The methylated metabolite mediated the greatest inhibitory effect and the glucuronidated metabolite inhibited the least. Analysis of potencies of inhibition of specific kinases, molecular modelling, and internalisation studies will attempt to determine how these compounds affect platelet signalling.

## 011 CORRELATION BETWEEN BAROREFLEX SENSITIVITY AND RESPIRATORY SINUS ARRHYTHMIA IN MYOCARDIAL INFARCTION PATIENTS

K. K. Deepak, K. Madan, A. Jaryal, A. Roy, N. Pandit, D. Prabhakaran, R. M. Pandey, S. K. Reddy. *Departments of Physiology, Cardiology and Nuclear Medicine, All India Institute of Medical Sciences, New Delhi, India*

**Rationale:** The vagal efferents to the heart are modulated by the baroreflex and respiratory sinus arrhythmia. Acute myocardial infarction (AMI) is associated with changes in the autonomic control of the heart including changes in baroreflex sensitivity and E:I ratio during a deep breathing test (DBT). This study investigated the association between baroreflex sensitivity and respiratory sinus arrhythmia as detected by the expiration to inspiration (E:I) ratio during DBT.
**Methods:** 32 patients (age 49.9$\pm$9.0 years) were studied 2 weeks after AMI. The baroreflex sensitivity was measured by the sequence method. The beat-to-beat blood pressure was monitored non-invasively using Finapres along with ECG. The DBT was carried out at 6 cycles/min and the E:I ratio was calculated as the ratio of maximum RR interval during expiration and the minimum RR interval during inspiration. The data were analysed by Spearman's test.
**Results:** The median BRS was 14.74 (range 0.00 to 36.86) and the median E:I ratio was 1.19 (1.04 to 1.52). The E:I ratio correlated positively with BRS calculated from the diastolic blood pressure ($r=0.395$, $p=0.028$); however, such a relation only existed for BRS related to rising sequences of systolic blood pressure and falling sequences of diastolic blood pressure. The E:I ratio also showed a positive correlation with the number of sequences for diastolic ($r=0.504$, $p=0.004$) and systolic blood pressure ($r=0.504$, $p=0.004$).
**Conclusions:** Respiratory sinus arrhythmia and the baroreflex are two important physiological responses that modulate vagal activity in the heart. In patients with AMI, values of BRS were positively correlated with respiratory sinus arrhythmia, indicating a complex interaction between vascular, respiratory, and cardiac controls. The lack of a positive correlation between BRS and respiratory sinus arrhythmia related to systolic fall and diastolic rise in blood pressure suggests there are selective disturbances in BRS.

## 012 THALLIUM-201 POSITIVE PATIENTS WITH CORONARY ARTERY DISEASE SHOW HIGH HEART RATE VARIABILITY

A. K. Jaryal, R. Khadka, R. Narang, C. D. Patel, K. K. Deepak. *Departments of Physiology, Cardiology, and Nuclear Medicine, All India Institute of Medical Sciences, New Delhi, India*

**Background:** Heart rate variability (HRV) is altered in patients suffering from angina. Angina patients have either positive or negative scintigraphic findings on thallium-201 (Tl-201) myocardial perfusion SPECT. We studied whether HRV differed in these two groups of patients.
**Methods:** Thallium-201 SPECT and short term HRV were assessed in 24 patient with anginal symptoms after stopping $\beta$ blockers for 48 hours. On the basis of Tl-201 SPECT the patients were divided into two groups: group I with stress induced ischaemia (n=14, age 56.21$\pm$8.84 years), and group II with no evidence of stress induced ischaemia (n=10, age 52.6$\pm$9.56 years). For HRV analysis, a 5 min ECG was recorded in all patients in the supine position and analysed in the time domain and the

frequency domain. The Kruskal–Wallis test was used for statistical analysis. Data are expressed as median (interquartile range); $p<0.05$ was considered significant.
**Results:** In group I, the time domain parameters (SDNN, 41.96 (35.42 to 57.83) *vs* 33.46 (16.61 to 35.56), $p=0.010$; RMSSD, 34.94 (22.66 to 50.02) *vs* 17.1 (9.56 to 21.72), $p=0.0028$; and pNN50, 7.17 (2.47 to 11.44) *vs* 0.28 (0 to 1.43), $p=0.002$), which are considered markers of parasympathetic tone, were significantly higher than in group 2. Similarly, in the frequency domain analysis, total power (2506.16 (1403.5 to 3080.34) *vs* 663.46 (308.77 to 1278.73), $p=0.003$), HF power (622.63 (226.51 to 972.82) *vs* 165.78 (47.68 to 221.33), $p=0.003$), and LF power (473.29 (332.2 to 677.69) *vs* 159.68 (134.04 to 369.04), $p=0.019$) were higher in group I.
**Conclusions:** Patients with anginal symptoms having documented evidence of ischaemia on Tl-201 SPECT had greater HRV, showing increased parasympathetic tone compared with those presenting with anginal symptoms but normal Tl-201 SPECT.

## 013 HYPERBARIC OXYGEN PRECONDITIONING PROMOTES CARDIOPROTECTION FOLLOWING ISCHAEMIC REPERFUSION INJURY BY IMPROVING MYOCARDIAL FUNCTION, LIMITING NECROSIS, AND ENHANCING THE INDUCTION OF HSP72

J. Z. Yogaratnam, G. Laden, J. Greenman, A. M. Seymour, A. Cale, S. Griffin. *Department of Biological Sciences, University of Hull, UK*

**Background:** Ischaemic reperfusion injury (IRI) occurs during coronary artery bypass graft surgery (CABG). Hyperbaric oxygen (HBO) post-IRI is known to limit myocardial damage. In this study we assessed the cardioprotective effects of HBO clinically (HBO preconditioning) during CABG.
**Methods:** This randomised control study of patients having CABG using cardiopulmonary bypass (CPB) consisted of 40 patients in group A (control group) and 41 in group B (HBO group). HBO preconditioning was carried out 4 h before CABG for 90 min at 2.4 ATA using 100% oxygen. Venous blood was taken pre-HBO, 5 min after onset of CPB, 5 min post-IRI, and 2 and 24 h post-CPB. Right atrial biopsies were taken post-induction, 5 min after onset of CPB, 5 min post-IRI, and 5 min post-CPB. Blood sera and biopsy protein extracts were analysed using a quantitative sandwich ELISA for troponin-T and Hsp72. Haemodynamic measurements, using a pulmonary artery catheter, were taken post-induction and 5 min, 2, 4, 8, 12, and 24 h post-CPB.
**Results:** Post-CPB, group B showed significantly greater increases than group A in stroke volume (SV) ($p=0.01$), left ventricular stroke work (LVSW) ($p=0.005$), and left ventricular stroke work index (LVSWI) ($p=0.02$). Myocardial Hsp72 ($p=0.09$) and serum troponin-T ($p=0.7$) did not differ between the groups. However, following IRI, Hsp72 fell in group A but rose in group B, and there was a smaller rise in the quantity of serum troponin-T in group B.
**Conclusions:** HBO preconditioning before IRI improves myocardial SV, LVSW, and LVSWI. It also appears to preserve the myocardium by limiting myocardial necrosis and the release of troponin-T. Furthermore, as it enhances the synthesis of myocardial Hsp72 following IRI, this suggest that HBO preconditioning is capable of inducing a myocardial protective mechanism that leads to protein repair and improved function following IRI.

## 014 MYOSTATIN: A POTENTIAL THERAPEUTIC TARGET FOR TREATING HEART FAILURE?

G. Mckoy, K. A. Bicknell, K. Patel, G. Brooks. *Schools of Pharmacy and Biological Sciences, University of Reading, UK*

Heart muscle cells lose their ability to proliferate soon after birth and grow instead by increasing in size (hypertrophy). Although hypertrophy is a normal adaptive response to stress, it can become maladaptive, leading to heart failure. Mature cardiomyocytes are unable to regenerate but they increase cardiac mass by hypertrophic growth. This has serious consequences for patients with heart failure after myocardial infarction. The transition from hyperplastic to hypertrophic growth in myocytes is mediated through differential expression and activities of specific cell cycle proteins. Thus the characterisation and manipulation of molecules that regulate expression of critical genes involved in cardiomyocyte proliferative and hypertrophic growth might lead to the identification of therapeutic targets that either promote adult myocyte proliferation or limit pathological hypertrophic growth. We have shown that myostatin, a potent regulator of skeletal muscle growth and proliferation, is expressed in cardiomyocytes. Our in vitro studies demonstrate that myostatin significantly inhibits serum induced proliferation of rat fetal (E18) and neonatal (P0) cardiomyocytes by 50–60% relative to untreated controls. Flow cytometric analysis showed that this inhibition occurs mainly through a block in the G1 to S phase transition of the cardiomyocyte cell cycle. Immunoblot analysis demonstrated that the cell cycle blockade coincided with the altered expression of key cell cycle proteins p21 and CDK. In addition recombinant myostatin inhibited the phenylephrine induced hypertrophic response in non-proliferating cardiomyocytes such that myostatin inhibited the increase in cell size as well as the induction of fetal genes, ANF, and sarcomeric $\alpha$ actin, characteristic of cardiac hypertrophic responses that progressively lead to heart failure. Taken together the ability of myostatin to modulate cardiomyocyte growth identifies it as a potential target for treatment of heart failure.

## 015 NON-GENOMIC SIGNALLING OF THE RETINOIC X RECEPTOR THROUGH INHIBITION OF GQ SIGNALLING IN HUMAN PLATELETS

L. A. Moraes[1], D. Bishop-Bailey[2], J. M. Gibbins[1]. [1]*School of Biological Sciences, University of Reading, UK;* [2]*William Harvey Research Institute, Bart's and the London, Queen Mary's School of Medicine and Dentistry, London, UK*

The retinoid X receptors (RXR) are important transcriptional nuclear hormone receptors, acting either as homodimers or as binding partners for at least one quarter of all the known human nuclear receptors. Functional non-genomic effects of nuclear receptors are poorly understood; however, peroxisome proliferator activated receptor (PPAR)$\gamma$, PPAR$\beta$, and the glucocorticoid receptor have recently been found to be present in human platelets. Human platelets express RXR$\alpha$ and RXR$\beta$. In this study we report that RXR ligands inhibit platelet aggregation and TXA2 release in response to ADP and the thromboxane mimetic U46619, but inhibit only weakly the platelet response to collagen. ADP and TXA2 both signal through the heterotrimeric G-protein Gq. RXR rapidly binds Gq but not Gi/z/o/t/gust in an RXR ligand dependent manner and inhibits both Gq induced mobilisation of calcium from intracellular stores and Rac activation. We propose that RXR ligands may have beneficial clinical actions through inhibition of platelet activation. Furthermore our results demonstrate a novel non-genomic mode for nuclear receptor action, and an important functional crosstalk between G-protein and nuclear receptor signalling families.

This work was supported by the British Heart Foundation and Bart's and the London, Queen Mary's School of Medicine and Dentistry.

## 016 OVEREXPRESSION OF NF-κB P50 INHIBITS LPS INDUCED CYTOKINE SECRETION BY MACROPHAGES AT LOW PH: IMPLICATIONS FOR THE RESOLUTION OF INFLAMMATION IN ATHEROSCLEROSIS

A. B. Gerry, D. S. Leake. *School of Biological Sciences, University of Reading, UK*

**Objectives:** Atherosclerosis is a chronic inflammatory disease in which the master inflammatory transcription factor NF-κB is activated. Plaques that are vulnerable to rupture, leading to thrombosis, myocardial infarction, and strokes, have a higher degree of inflammation than stable plaques, and have more lipid-rich areas of low extracellular pH. We have shown that low pH increases and prolongs pro-inflammatory p65:p50 binding to the NF-κB promoter sequence. NF-κB p50 homodimers antagonise p65:p50 binding. We therefore set out to modulate NF-κB signalling to inhibit pro-inflammatory NF-κB signalling at low extracellular pH.
**Methods:** Human p50 was overexpressed in THP-1 monocytes and human peripheral blood monocyte derived macrophages, followed by incubation for 24 h at pH 7.4 or pH 7.0 before stimulation. NF-κB p50 expression was assessed by western blots and confocal microscopy. DNA binding was assessed by electrophoretic mobility shift assay and cytokine secretion was measured by ELISA.
**Results:** Adenovirus mediated overexpression of p50 increased p50:p50 DNA binding, inhibited LPS induced proinflammatory tumour necrosis $\alpha$ and interleukin 6 secretion, and increased anti-inflammatory interleukin 10 secretion, especially at low extracellular pH.
**Conclusions:** Overexpression of p50 sufficient to allow the formation of p50 homodimers may inhibit inflammatory cytokine secretion in areas of low extracellular pH where p65:p50 signalling is increased. Modulation of p50 expression may therefore be a therapeutic approach to aid resolution of inflammation in atherosclerosis and other diseases.

Funding: British Heart Foundation.